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On 18 Dec. 2002

TOWNSEND and TOWNSEND and CREW LLP

By: Malinda Adajt

**PATENT**  
Attorney Docket No.: 02307O-067720US  
Client Ref. No.: 80-215-3

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## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

LALEH SHAYESTEH et al.

Application No.: 08/905,508

Filed: August 4, 1997

For: GENETIC ALTERATIONS  
ASSOCIATED WITH CANCER

Examiner: Lisa B. Arthur

Art Unit: 1655

Declaration of Joe W. Gray, Ph.D. under 37  
C.F.R. §1.132

Assistant Commissioner for Patents  
Washington, D.C. 20231

1. I, Joe W. Gray, am a Professor of Laboratory Medicine and Radiation Oncology at the University of California San Francisco. I am a co-inventor of the subject matter disclosed and claimed in the above-referenced patent application.

2. I received a Ph.D. in Physics in 1972 from Kansas State University. My field of expertise is cancer and cytogenetics. I have been in this field for over 25 years and have authored over 250 publications in this area.

3. I have read and am familiar with the contents of the subject patent application. I have also reviewed the reference Hu *et al. Clin. Cancer Res.* 6:880-886, 2000, which was submitted to the Examiner in Applicants' response mailed October 1, 2001. The paper was presented to provide additional evidence to show that administration of

phosphatidylinositol 3-kinase (PI3-K) inhibitors inhibit ovarian cancer cell proliferation *in vivo*. It is my understanding that the Examiner is requiring that at least one of the inventors of this application explain the relevant parts of the reference. This Declaration is therefore presented to explain the studies presented in the Hu *et al.* reference.

4. Athymic immunodeficient mice were used in a model of ovarian carcinoma to examine the effects of administration of a PI3-K inhibitor on the growth of ovarian cancer cells. This model closely resembles stage 3 ovarian epithelial carcinoma, with both extensive dissemination of ovarian carcinoma cells to peritoneal surfaces and the development of massive ascites. Mice were inoculated with cells of the ovarian cancer cell line OVCAR-3, in which the gene encoding the P110 $\alpha$  catalytic subunit of PI3-K is increased in copy number. Eight days after inoculation, one group of mice was treated with the PI3-K inhibitor LY294002 for 21 days. The control group received the vehicle only. Body weight and abdominal circumference were measured twice weekly. At the end of the experiment (28 days after inoculation with the cancer cells), the tumors in the control and treated animals were evaluated.

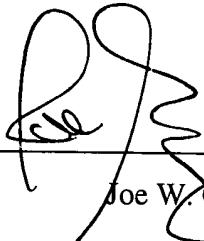
5. Because intraperitoneal tumor growth and the development of ascites could not be measured directly during the experiment, body weight and abdominal circumference, which reflect increasing ascites accumulation and tumor burden, were measured. The results (Fig. 3, page 883) showed that abdominal circumference significantly increased in the control animals compared to the animals treated with PI3-K inhibitor.

6. Tumor burden was also assessed at postmortem examination. The results showed that the mean tumor burden in the treated group was reduced by 65% compared to the control group. Virtually no ascites developed in the treatment group. *In vivo* morphological studies also demonstrated that treatment with PI3-K inhibitor induced marked nuclear pyknosis and diminished cytoplasmic volume in the tumor cells (Fig. 2, page 883), which is indicative of apoptosis.

7. Thus, these experiments show that PI3-K inhibitor significantly inhibits growth and ascites formation of ovarian carcinoma *in vivo*.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 12/13/02 By \_\_\_\_\_



Joe W. Gray, Ph.D.